



RESEARCH ARTICLE

Inheritance of fertility restoration in A4 cytoplasm of Pigeonpea (*Cajanus cajanifolius* L.)

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Abstract

A set of three medium-duration CMS lines ICPA 2043, ICPA 2047 and ICPA 2048 were used in study to investigate fertility restoration by making twenty-eight test crosses at the Department of Pulses, TNAU, Coimbatore. ICPA 2047 exhibited fertility restoration ranging from 0-40 % with negligible pod setting except for the cross ICPA 2047 × ICP 3181. In contrast, sixteen F₁s derived from ICPA 2048 showed pollen fertility ranging from 10-48 % except for the cross ICPA 2048 × ICP 9224. Similarly, sixteen F₁s derived from ICPA 2043 demonstrated 15-38 % pollen fertility, with ICPA 2043 × LRG 41 showing 98 % fertility and good pod setting. Among the hybrids, ICPA 2043 × LRG 41 exhibited high fertility, ranging from 95.58 % to 98.67 % over two years. This suggests that dominant fertility-restoring genes from the restorer parent were incorporated into the hybrid, which showed consistent fertility restoration across different years. In the current investigation, the restorer line ICP 3181 demonstrated monogenic inheritance of fertility restoration in crosses with ICPA 2047 and ICPA 2048, following 3:1 ratio. Conversely, the cross ICPA 2043 × LRG 41 exhibited a digenic duplicate dominance inheritance pattern. These results suggest that the fertility restoration in pigeonpea can be inherited monogenically or digenically, depending on the hybrid. The identified hybrids, particularly ICPA 2043 × LRG 41 demonstrate significant potential for improving fertility restoration and enhancing productivity in pigeonpea breeding programs.

Keywords

A4 cytoplasm; fertility restoration; inheritance; male sterile lines; pigeonpea

Introduction

To address the stagnant productivity of the global pigeonpea crop, caused by various biotic and abiotic factors as well as a lack of high-yielding varieties over the past three decades, heterosis breeding has been employed in pigeonpea breeding research over 60 years ago. Increasing pigeonpea yield has been the primary goal of the national breeding efforts (1). The only way to achieve yield maximisation in this frequently cross-pollinated exception (due to insect pollination) among the grain legumes of the world was assumed to be through the exploitation of hybrid vigour. Any crop must have stable male sterile lines as the female parents and stable restores as the pollen parents in order to generate good-yielding hybrids. Fertility restorer (rf) genes suppress male sterility, enabling the use of CMS systems for hybrid seed

production. In pigeonpea, there are several cytoplasmic nuclear male sterility (CMS) systems.

Despite the introduction of several hybrid varieties, farmers' fields do not experience significant yield improvements. This is due to factors such as partial fertility restoration and high genotype-environment interactions. Most morphological characteristics of *C. cajanifolius* A4 cytoplasm are similar to those of cultivated varieties (2). According to reports, the CMS lines that contain A4 cytoplasm are extremely stable over a wide range of conditions and periods without displaying any morphological abnormalities. The current study presents a comparative evaluation of fertility restoration in hybrids containing A4 cytoplasm and discusses the importance of these findings for pigeonpea hybrid breeding. From *C. cajanifolius*, three CMS lines namely, ICPA 2047, ICPA 2048 and ICPA 2043 were utilised. Even though these CMS lines were recorded, differences were found in pollen fertility restoration, which may be the result of different intergenomic and cytoplasmic genome interactions between the cultivars that were converted to the A-line and the wild source cytoplasm (3). Understanding restorer genetics is crucial for developing new restorers for available CMS lines. Consequently, the goal of the current inquiry is to learn how CMS lines descended from A4 origins.

Materials and Methods

A set of three CMS lines [1] ICPA 2043, ICPA 2047 and ICPA 2048, were used for the current study. To attain the higher yield in the A4 cytoplasm the medium maturity group for fertility restoration with proper stability these CMS lines were used for the current study. These CMS lines were used to create 28 test crosses. At the Department of Pulses, TNAU, Coimbatore, 28 test crosses using germplasm lines were made during *khariif* 2016 (Table 1). F1 seeds were separately gathered and produced during the 2017 *khariif* season. All of the CMS lines and testers were planted inside the nylon net at the Department of Pulses, CPBG, TNAU, Coimbatore in order to shield the experimental materials from pollinating insects. In order to prevent pollen shedders during hybridisation, individual CMS line plants were tested for male sterility.

At flowering, new pollen from the 28 crossings in a Line × Tester mating strategy was used to manually pollinate the male-sterile lines on more than 500 occasions. With a mean crossing

success of 60 %, 40-80 pods were collected from each cross. All the hybrid combinations were planted during *khariif* 2018 in an unreplicated state to examine the stability of fertility restoration. Two seeds per hill were planted with the recommended spacing of 90 × 30 cm and this produced a 90% plant stand. To raise a robust crop, conventional cultural practices were used. Five completely developed but unopened flower buds were chosen randomly from each cross of 30 plants and their anthers were crushed in a 2 % acetocarmine solution to examine the pollen fertility. Three microscopic fields [3] were examined under a light microscope to examine the pollen productivity of each plant. The pollen grains that were completely dyed were regarded fertile, whilst the pollen grains that were empty or only partially stained were thought to be male-sterile. The plants were divided into three groups according to (4) with some modifications: fertile (>90 % pollen fertility with good pod setting), partial fertile (11-89 % pollen fertility with poor pod setting) and sterile (0-10 % pollen fertility with negligible pod setting). A measure of fertility restoration was the proportion of male fertile plants in each F1 hybrid. Based on the pollen fertility status of the twenty-eight test crosses, three hybrids from ICP 3181, ICP 9224 and LRG 41 (exhibiting >80 % pollen fertility restoration and good pod setting) were chosen and their morphological traits were used as descriptors to identify the true parent as well as F₁s. In order to obtain seeds for the F₂ generation, all of the F1 plants were selfed using insect-proof nylon net cages. They were then crossed [1] to their respective A-lines to produce BC₁ seeds and three new crosses were also made in 2016. A Chi-square test was used to evaluate the goodness of fit to various predicted ratios in the F₂, BC₁F₁ and test cross generations.

Results and Discussion

A stable CGMS (cytoplasm Male Sterility System) and effective restorers are crucial for developing CGMS hybrids in pigeonpea (2). The confirmation of potential fertility restorers cannot be done based on pollen fertility alone due to the stability of restorers. However, the studying pod set through self-pollination and open pollination under isolation is considered as an essential criterion for identification of restorer in pigeonpea (5). Consistent with earlier research (6) at Patancheru, test crosses on male sterile plants following hand pollination showed high pod setting success (60 %) (Table 2). This demonstrates that a plant

Table 1. CMS lines used for crossing in medium duration

S.No.	CMS lines	Source	Plant type	Days to 50% flowering	Anther morphology	Mean pollen fertility (%)
1	ICPA 2047	A ₄	NDT	115	Yellow, scaly	0.0
2	ICPA 2048	A ₄	NDT	120	Yellow, scaly	0.0
3	ICPA 2043	A ₄	NDT	114	Yellow, scaly	0.0
List of test crosses made from the A4 CMS lines						
1.	ICPA 2047 × ICP 525403			16	ICPA 2047×ICP 2047	
2.	ICPA 2047 × ICP 525454			17	ICPA 2048 × ICP 525403	
3.	ICPA 2047 × ICP 545457			18	ICPA 2048 × ICP 525454	
4.	ICPA 2047 × ICP 9224			19	ICPA 2048 × ICP 525457	
5.	ICPA 2047 × ICP 10697			20	ICPA 2048 × ICP 9224	
6.	ICPA 2047 × ICP 10788			21	ICPA 2048 × ICP 10788	
7.	ICPA 2047 × ICP 525438			22	ICPA 2048 × ICP 525438	
8.	ICPA 2047 × ICP 525440			23	ICPA 2048 × ICP 525521	
9.	ICPA 2047 × ICP 525471			24	ICPA 2048 × ICP 12321	
10.	ICPA 2047 × ICP 525421			25	ICPA 2048 × ICP 3181	
11.	ICPA 2047 × ICP 12321			26	ICPA 2048 × ICP 3666	
12.	ICPA 2047 × ICP 1135			27	ICPA 2043 × ICPB 2043	
13.	ICPA 2047 × ICP 2224			28	ICPA 2043 × LRG 41	
14.	ICPA 2047 × ICP3181					
15.	ICPA 2047 × ICP 3666					

Table 2. Description of % success crossed seeds harvested through manual pollination

S.No.	Crosses	Bud pollinated	Seeds harvested	% success
F₁ Hybrids				
1	ICPA 2047 / ICP 3181	94	51	54.25
2	ICPA 2048 / ICP 9224	95	57	60.00
3	ICPA 2043 / LRG 41	118	67	56.77
Test crosses				
4	ICPA 2047// ICP 2047/ ICP 3181	194	84	43.29
5	ICPA 2048/ ICP 2048/ ICP 9224	188	69	36.70
6	ICPA 2043/ ICP 2043/ LRG 41	254	125	49.21

could set a large number of pods even with only a small percentage (10 % or more) of viable pollen grains.

Among the twenty-eight F₁ hybrids produced by the CMS line ICPA 2047 from sixteen crossings, only one cross, ICPA 2047 × ICP 3181, showed significant seed setting at 43.29 %. The rest exhibited minimal pod setting.

Among the ten hybrids derived from ICPA 2048 × ICP 9224, showed notable seed setting with 36.70 %.

For ICPA 2043 × LRG 41, two F₁s descended from ICPA 2043 revealed 49.21 % of seed setting. The test cross between ICPA 2043 and LRG 41, one of the twenty-eight hybrids produced from the three CMS lines ICPA 2047, ICPA 2048 and ICPA 2043, displays 98 % fertility and good pod setting (Table 3). These crosses were subsequently assessed in isolation in *kharif* 2017 and obtained good pod setting because of restoration ability.

All of the plants in the three ICPA 2043 × LRG 41 hybrids were showing fertile during the course of two years, *kharif* 2017 and *kharif* 2018. (Table 3). This demonstrates that the hybrid successfully integrated dominant fertility-restoring genes from the restorer parent and that the hybrid had shown excellent fertility restoration stability over two years. This hybrid may be tested over years in different locations will lead to the potential application of the knowledge in the hybrid pigeonpea breeding programme.

LRG 41 stood out among the testers and displayed perfect male sterility preservation and fertility restoration in the A4 cytoplasm, whereas ICP 3181 and ICP 9224 also had fertility

Table 3. Fertility restoration of the Hybrid ICPA 2043 × LRG 41 for two consecutive years

Tester		ICPA 2043	
		Total plants	Fertility restoration (%)
LRG 41	Dept. of Pulses, <i>kharif</i> 2017	120	94
	Dept. of Pulses, <i>kharif</i> 2018	240	98
	Mean (across two Years)	180	96

Table 4. Segregation ratios for male fertile and sterile plants in F₁ and F₂ generations of crosses involving A4 CMS lines and fertility restorers in Pigeonpea

Cross	Generation	Number of plants				Chi-square probability
		Total	Fertile	Sterile	Ratio	
ICPA 2047 × ICP 3181	F ₁	16	16	0	-	-
	F ₂	340	248	92	3:1	0.98
ICPA 2048 × ICP 9224	F ₁	15	15	0	-	-
	F ₂	315	290	25	3:1	0.99
ICPA 2043 × LRG 41	F ₁	17	17	0	-	-
	F ₂	421	387	34	15:1	0.99

Pollen fertility of testcross populations						
S.No.	Test crosses	No. of plants			Segregation ratio	
		Total	Fertile	Partial fertile		Sterile
1	ICPA 2047// ICP 2047/ ICP 3181	96	68	0	28	X ² 3:1=2.78 ^{NS}
2	ICPA 2048// ICP 2048/ ICP 9224	95	62	0	33	X ² 3:1=3.14 ^{NS}
3	ICPA 2043// ICP 2043/ LRG 41	111	94	0	17	X ² 3:1=3.74 ^{NS}

Non-significant at p=0.05 & 0.01 level respectively

restoration with a range of 80-85 %. In the experiment, it was discovered that the CMS lines ICPA 2047 and ICPA 2048 (A4 cytoplasm) could not maintain male sterility without restorers, namely ICP 3181 and ICP 9224. Only one line, ICPA 2043 (A4) cytoplasm, retained perfect male sterility out of twenty-eight crosses comprising three CMS lines, hence ICPA 2023 may be used with LRG 41 may be resorted to fertility. 70-78 % of the male viable plants in the remaining crosses with ICPA 2047 and ICPA 2048 are male.

The successful transmission of restorer genes between genotypes is governed by the underlying genetics of fertility restoration, which is crucial for hybrid breeding programs. This study revealed that the restorer ICP 3181 exhibited monogenic inheritance (3:1) when crossed with ICPA 2047, a trait similarly observed in crossed with ICPA 2048 (Table 4). The fertility restoration of in hybrids, ICPA 2047 × ICP 3181 and ICPA 2048 × ICP 9224 was controlled by a single dominant gene based on the F₂ value. In contrast, the hybrid ICPA 2043 × LRG 41 exhibited a di-genic duplicate dominance inheritance of fertility restoration (Table 4). A study was conducted on the inheritance of fertility restoration in three diverse medium-duration CMS lines of pigeonpea (7). Various cytoplasmic sources with the same set of male parents expressed fertility restoration differently (7). Research indicates that one or two fertility-recovering genes were in charge of restoring fertility in the A4 CMS lines of pigeonpea (8). In soyabean, it was found that the monogenic to digenic gene in CMS lines of NJCMS2 A controlled fertility restoration (8).

The variable responses of CMS lines with the same cytoplasm in test crosses may result from the accumulation of recessive nuclear genes from recurrent male ancestors during backcrossing. The accumulation might lead to changes in the cytoplasmic genes governing male sterility (9). The presence of modifier genes that affect the expression and penetrance of fertility-restoring genes may also contribute to differences in the segregation pattern (10). In the current study, the nuclear background of male-sterile and fertility-restoring lines had an impact on the inheritance of male fertility restoration in CMS lines of pigeonpea. 30 male sterile lines from *Zea mays* (maize) were divided into several groups based on their ability to restore fertility (1). Due to the limited number of CMS lines in this study, grouping based on fertility restoration patterns was not feasible, potentially limiting the breadth of conclusions regarding genetic variability. The fertility of hybrids between the same female and particular males in sorghum (*Sorghum vulgare*) varied, according to (11). Similarly, the variation in fertility restoration observed in

the current study could be attributed to the presence or absence of key fertility genes. Variations in fertility restoration observed in the current study could be attributed to the presence or absence of fertility genes (12). Variations in sunflower fertility restoration have been reported using several testers and the same cytoplasm (*Helianthus annuus* L.) (13). Studies at ICRISAT (International Crops Research Institute for the semi-arid Tropics) revealed the presence of one to three dominant genes for restoring male fertility in all CMS sources of pigeonpea.

Conclusion

According to the research, ICPA 2043 from A4 cytoplasm is a consistent male sterile line throughout a number of seasons because of genetic uniformity and environmental adaptability. The LRG 41 variety demonstrates good fertility restoration, unlike cultivars ICP 3181 and ICP 9224, which exhibited variability in fertility restoration among testers. This inconsistency may be attributed to genetic impurities in the male parents, potentially resulting from spontaneous outcrossing and difficulties in maintaining genetic stocks under natural pollination. Understanding the inheritance pattern of fertility restoration facilitated the development of new restorers for the same cytoplasmic source. The differences in behaviour of the three fertility restorer lines ICP 3181, ICP 9224 and LRG 41 were attributed to the interactions of the three female parents' different nuclear genes. The pigeonpea variety LRG 41 was identified as a stable restorer, having significant implications for future breeding programs.

Future directions

Hybrid pigeonpea, with its potential for higher yields and improved quality, can play a vital role in meeting this demand and ensuring food security. In conclusion, hybrid pigeonpea has demonstrated substantial potential for yield enhancement, offering numerous benefits to both farmers and the broader agricultural industry. Continued research, for fertility restorer development and promotion of these hybrids can pave the way for a more productive and sustainable future in pigeon pea cultivation.

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Authors' contributions

ATH has contributed to writing the original draft, reviewing and editing, methodology, data curation and conceptualization. SK has done review and editing and data curation. MS contributed through review and editing, data curation and methodology. WG conducted formal analysis. MKM contributed to formal analysis, review and editing. SM Samyuktha also conducted formal analysis, review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical issues: None

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